

Appl. No. 09/744,384
Amendment dated January 30, 2004
Reply to Office Action of September 24, 2003

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Previously presented) A method of generating a cell culture comprising dopaminergic neuron cells, said method comprising the sequential steps of:
 - a. providing precursor cells comprising human or rat fetal central nervous system cells;
 - b. proliferating precursor cells, said step of proliferating comprising:
 - i. incubating a suspension of said precursor cells in a proliferating medium which includes basic fibroblast growth factor (bFGF) to form proliferated precursor cells; and subsequently
 - c. differentiating said precursor cells, said step of differentiating comprising:
 - i. incubating said precursor cells in an incubation vessel which contains differentiation medium in a manner effective to form a reaggregation of differentiated dopaminergic neuron cells that is not adhered to any surface of the incubation vessel, wherein the differentiation medium includes ascorbic acid.
2. (Original) The method of claim 1, wherein said step of differentiating comprises incubating the precursor cells in a roller tube.
3. (Original) The method of claim 1, wherein said step of proliferating comprises plating said suspension of precursor cells onto a proliferating medium and incubating for 5 to 10 days.
4. (Original) The method of claim 3, wherein said suspension comprises 50×10^3 cells/ml to 500×10^3 cells/ml precursor cells.

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5. (Original) The method of claim 1, wherein said step of differentiating comprises incubating said ^{proliferated} precursor cells in differentiation medium for 5 to 10 days.
6. (Original) The method of claim 1, wherein said cell culture comprises between 1% and 5% glial cells.
7. (Original) The method of claim 1, wherein said precursor cells comprise mesencephalic cells.
8. (Original) The method of claim 1, wherein said cell culture further comprises cholinergic neuronal cells.
9. (Original) The method of claim 8, wherein said precursor cells comprise basal forebrain cells or spinal cord cells.
10. (Original) The method of claim 1, wherein said cell culture further comprises serotonergic cells.
11. (Original) The method of claim 10, wherein said precursor cells comprise nucleus raphe cells.

12-17. (Cancelled)

- 12/18. (Currently Amended) A cell culture comprising a population of cells;
about 80% to about 95% of ~~a total cell~~ the population of cells being in the culture
~~comprise~~ differentiated neuronal cells; and
less than 5% of the total cell population of cells being ~~comprises~~ glial cells; and
at least 9.9% of wherein the differentiated neuronal population of cells being
~~comprise~~ dopaminergic cells.

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- 13 ~~19~~ (Currently Amended) The cell culture of claim ~~18~~¹², wherein ~~dopaminergic neurons~~ comprise 18.4% +/-5.1% of the total cell population is dopaminergic neurons.
- 14 ~~20~~ (Previously presented) A method of treating a patient for Parkinson's disease, said method comprising administering cells produced according to the method of claim 1 to the patient to treat the patient for Parkinson's disease.
21. (Cancelled)
- 15 ~~22~~ (Previously presented) The method of claim ~~20~~¹⁴, wherein the method of treating a patient further comprises:
- suspending said cells in a physiologically compatible carrier;
 - introducing a therapeutically effective amount of said cells into the brain of the patient.
- 16 ~~23~~ (Previously presented) The method of claim ~~22~~¹⁵ wherein introducing a therapeutically effective amount further comprises administering $1-4 \times 10^6$ dopaminergic neurons, wherein administering further comprises loading said cells into a syringe and injecting them within the parenchyma of the patient's brain.
- 17 ~~24~~ (Previously presented) An assay for evaluating the effect of substances on differentiated neuronal cells, comprising:
- culturing differentiated neuronal cells, said step of culturing comprising:
 - proliferating neuronal precursor cells, said step of proliferating comprising:
 - incubating said neuronal precursor cells in proliferating medium which includes basic fibroblast growth factor (bFGF); and
 - differentiating said neuronal precursor cells, said step of differentiating comprising:

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- aa. incubating said precursor cells in an incubation vessel which contains differentiation medium in a manner effective to form a reaggregation of differentiated cells that is not adhered to any surface of the incubation vessel, wherein said differentiating medium includes ascorbic acid,
- B. exposing said differentiated neuronal cells to the substance; and
- C. observing the effect of the substance on said differentiated neuronal cells.

25. (Cancelled)

18 ~~26~~. (Previously presented) The method of claim 1, wherein the precursor cells comprise human fetal cells obtained between about embryonic week 5 and about embryonic week 8.

19 ~~21~~. (Previously presented) The method of claim 1, wherein the precursor cells further comprise rat fetal cells obtained between about embryonic day 10 and about embryonic day 12.

28-30. (Cancelled)

20 ~~31~~. (New) The cell culture of claim ¹²~~18~~, wherein about 15% to about 20% of the population of cells are dopaminergic cells.

21 ~~32~~. (New) The cell culture of claim ¹²~~18~~, wherein about 15% of the population of cells are dopaminergic cells.

22 ~~33~~. (New) The cell culture of claim ¹²~~18~~, wherein 14.1 +/- 4.2% of the population of cells are dopaminergic cells.

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25 31. (New) The cell culture of claim ¹²18, wherein 9.9% to 23.5% of the population of cells are dopaminergic cells.